



Supplementary Figure 1

Diagram for CATCHA construct.



Supplementary Figure 2

Representative view of ebony (left) and non-ebony (right) F2 flies from experiments described in Fig. 1c.

	F0 #1	F0 #2	F0 #3	F0 #4	F0 #5	Total (#1 to #5)	<i>vas-cas9</i> /+ control
F2 <i>ebony</i> fly count	8	6	5	11	7	37	121
F2 non- <i>ebony</i> fly count	96	103	89	139	97	524	0
Fraction of non- <i>ebony</i>	92.3%	94.5%	94.7%	92.7%	93.3%	93.4%	0.0%

Supplementary Table 1. *ebony* phenotypic analysis for assessing CATCHA-induced *cas9* ablation.

Columns from F0 #1 to F0 #5 were from five experiments in parallel using five CATCHA stocks. Each CATCHA stock (F2 in **Fig. 1b**) originated from a single F1 male that is positive for CATCHA. In *vas-cas9*/+ control group, *vas-cas9*/+ flies were used as F1 females in experiment described in **Fig. 1c**, demonstrating the efficiency of *ebony* gRNA. All flies in the first column (highlighted in yellow) were molecularly genotyped in **Fig. 1c**, which revealed that the 8 *ebony* flies also carry either CATCHA or NHEJ-mediated indels. Thus, the fraction of non-*ebony* in F2 is likely an underestimate of ablation efficiency. The disruption of the *ebony* gene in these 8 flies may be caused by the following two factors. First, the maternal contribution of *cas9* mRNA and/or Cas9 protein can disrupt *ebony* in the zygote. Since *vas-cas9* is expressed in nurse cells, *cas9* mRNA and Cas9 protein can be deposited into fertilized eggs regardless of the latter's genotype. If CATCHA-mediated ablation occurs late in the germline, sufficient numbers of nurse cells may still carry functional *vas-cas9* and deposit maternal Cas9 to cleave *ebony* in the zygote. That 100% (rather than 50%) *vas-cas9*/+ progeny are *ebony* supports this interpretation.

Materials and Methods

Construction of CATCHA plasmid and verification

gRNA sequence targeting *cas9* was generated by annealing oligos *cas9_gRNA_F* (CTTCGGCTACGCCGGCTACATTGA) and *cas9_gRNA_R* (AAACTCAATGTAGCCGGCGTAGCC). The resulting product was ligated into BbsI-digested plasmid pU6-BbsI-chiRNA¹ (Addgene ID #45946), followed by transformation into TOP10 Chemically Competent *E. coli* (Life Technologies). Positive pU6-cas9-chiRNA colonies were confirmed by sequencing.

The homology arm (1042 bp) for the downstream of the *cas9* cleaved site were amplified by PCR using primers *cas9C_infus_F* (GGGGATCCACTAGTTGACGGCGGAGCCAGC) and *cas9C_infus_R* (TGGCGGCCGCTCTAGCTTTCTGGATGTCCTCT), from template plasmid pHsp70-Cas9¹ (Addgene ID #45945), followed by PCR purification (Qiagen). In-Fusion (Clontech) was performed to insert the product into vector pU6-cas9-chiRNA at the XbaI site. Transformation and colony confirmation were conducted as described above, yielding a pU6-cas9-chiRNA-cas9C plasmid.

The homology arm (1003 bp) for the upstream of the *cas9* cleaved site was amplified by PCR using primers *cas9_N_Kpn1F* (ATGGTACCGCAAGAAATTCAAGGTGCTG) and *cas9_N_Xho1R* (ATCTCGAGATGTAGCCGGCGTAGCCGTTCT). Products were purified and treated with KpnI and XhoI restriction enzymes (New England Biolabs). Purified products were then ligated with pU6-cas9-chiRNA-cas9C vector digested by KpnI and XhoI. The final *cas9N-pU6-cas9-chiRNA-cas9C* plasmid (CATCHA construct, **Supplementary Fig. 1**) was verified by sequencing. Plasmid sequence is available at Harvard Dataverse (<https://dataverse.harvard.edu/>), with the title “Cas9-Triggered Chain Ablation (CATCHA) sequence materials”.

Drosophila stocks

w[1118]; PBac{y[+mDint2]=vas-Cas9}VK00027² (Bloomington stock # 51324) was injected with the CATCHA construct. This strain also served as the allelic *vas-cas9* to test conversion efficiency of integrated CATCHA. Transgenic gRNAs against *wingless* and *ebony* were gifts from Phillip Port³.

CATCHA stocks were confirmed by PCR with primers *cas9_F_seq* (CTGAGCGCCTCTATGATC) and *pU6_R_seq* (AACTAGTGGATCCCCCG) (blue primers in Fig. 1a, left). A 705-bp band was yielded from CATCHA positive stock. *cas9_F_seq* (CTGAGCGCCTCTATGATC) and *cas9_R_seq* (TCTCATTCCCTCGGTCACGT) (black primers in Fig. 1a, right) were used to obtain a 1221-bp band from CATCHA positive (HDR) flies, and a 654-bp band from flies carrying NHEJ alleles. The NHEJ alleles were sequenced using *nest_cas9_R_seq* primer (TGGTCAGCTCGTTATACACGGTG). The sequencing results are deposited at Harvard Dataverse.

Supplementary References

1. S. J. Gratz *et al.*, Genome engineering of *Drosophila* with the CRISPR RNA-guided Cas9 nuclease. *Genetics* **194**, 1029-1035 (2013).
2. S. J. Gratz *et al.*, Highly specific and efficient CRISPR/Cas9-catalyzed homology-directed repair in *Drosophila*. *Genetics* **196**, 961-971 (2014).
3. F. Port, H. M. Chen, T. Lee, S. L. Bullock, Optimized CRISPR/Cas tools for efficient germline and somatic genome engineering in *Drosophila*. *Proc Natl Acad Sci U S A* **111**, E2967-2976 (2014).